

SUMMARY

Monoterpenes (C₁₀) are isoprenoids usually employed as aromatic additives, are important components of wine aroma and some of them have antimicrobial and health beneficial properties. An efficient microbial production of these metabolites could be an inexpensive and environmentally friendly alternative to current techniques: chemical synthesis or extraction from natural sources.

They are usually produced in plants from geranyl pyrophosphate (GPP), a common intermediate of isoprenoid pathway in microorganisms and higher organisms; what opens the possibility of producing them in yeasts as *Saccharomyces cerevisiae*, qualified as GRAS, and that has been widely employed for biotransformation and production of enzymes and metabolites of biotechnological interest.

In this work, the inherent ability of this yeast for heterologous production of monoterpenes (linalool, geraniol, etc.) capable to improve organoleptic and/or functional properties of certain foods either being used as additives or produced during the elaboration process (e.g. vinification) has been characterized and improved by metabolic engineering approaches. Among the most relevant results we can find the selection of wine strain T₇₃ because of its better ability to produce monoterpenes, and the improvement of this production by overexpressing a deregulated version of Hmg1p (catalyzes mevalonic acid synthesis, MVA) and *IDII* (encodes isopentenyl pyrophosphate isomerase, that catalyzes the isomerization of farnesyl pyrophosphate synthase -FPPS- substrates). In addition, characterization of the FPPS role in GPP availability for the *Clarkia breweri* linalool synthase resulted in a 50 times increased production in yeast strains with wild-type enzyme (encoded by *ERG20*) replaced by one modified in its active site (K197E), encoded by *erg20-2* allele; reaching a global improvement of 80 times the basal linalool production (14.51 ± 0.90 µg/L) when coexpressing *IDII* gene and *erg20-2* allele in linalool producing strains lacking endogenous FPPS activity (1144.68 ± 139.39 µg/L).

Geraniol producing strains (bearing *Ocimum basilicum* -sweet basil- *GES* gene that encodes geraniol synthase) derived from T₇₃ were included in microvinification assays and geraniol concentration was over its perception threshold and a metabolic dispersion to other monoterpene (linalool, nerol and citronellol) and aromatic esters (geranyl and citronellyl acetate) was also detected. Increased monoterpene production established in *IDII* overexpressing strains under laboratory conditions was reproduced in these assays, demonstrating that the modification of key steps of MVA pathway is a valid strategy to modulate and/or improve wine aroma.